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The Wnt/ß-catenin pathway has been extensively studied for its role in development and cancer. The canonical Wnt signal is transduced by ß-catenin, which acts as a transcriptional coactivator by associating with the Tcf/LEF family of transcription factors. It has been established that Wnt signaling regulates the self-renewal of normal stem cells in both the hematopoietic systems and the epidermis. In addition, constitutive activation of the Wnt pathway has been implicated in a number of epithelial cancers, possibly by promoting stem cell survival. However, the importance of this pathway in breast stem/progenitor cells has not yet been **Objective/Hypothesis:** The central hypothesis of this study is that the Wnt/ß-catenin pathway plays a critical role in mammary gland stem cell survival, and as a result promotes tumorigenesis and resistance to conventional therapies.

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Introduction:

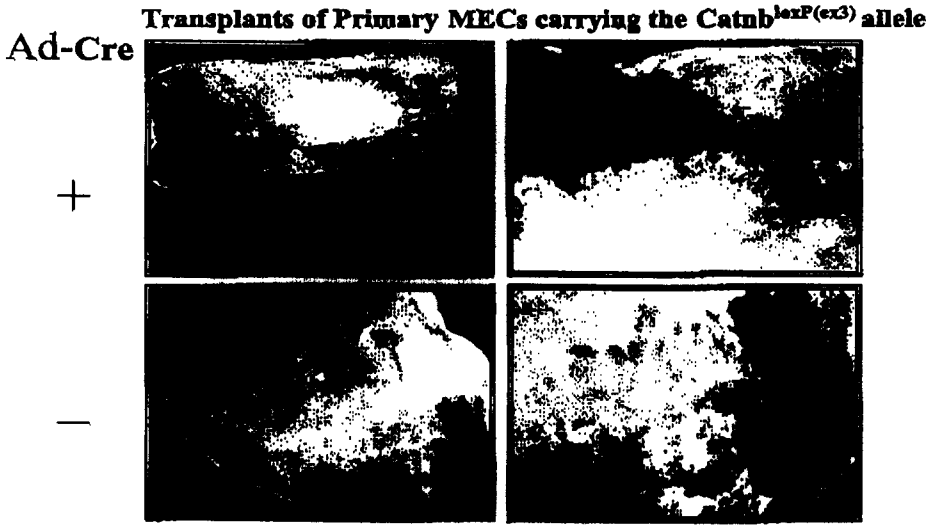
The Wnt/ß-catenin pathway has been extensively studied for its role in development and cancer. It has been established that Wnt/ß-catenin signaling regulates the self renewal of normal stem cells in the hematopoietic system, the epidermis, as well as many other organs, but the importance of this pathway in breast stem/progenitors has not yet been investigated. The overall objective of this study is to determine the mechanisms by which ß-catenin might promote breast stem/progenitor cell survival, while inhibiting mammary differentiation. If the Wnt/ß-catenin signal transduction pathway is critical for breast stem cell survival, it may be possible to sensitize these cells to chemotherapeutic agents by inhibiting this pathway. These studies may, therefore, provide new targets for understanding the etiology of, and be critical for the design of new treatments for, breast cancer.

Tasks:

- ❑ Task #1

1. To determine whether stabilizing ß-catenin in primary MECs will promote hamammry outgrowths using in vivo mammary gland reconstitution.

Figure 1.1.A

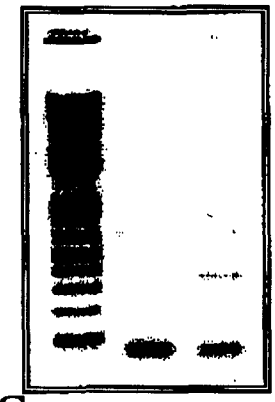


	# INJECTED	# OUTGROWTH
AD-CRE +	7	0/7
AD-CRE -	7	7/7

Transducing primary MECs in culture using Adenovirus driven Ad-Cre has been previously established in our lab (1). The primary MECs carrying the Catnb$^{lox(ex3)}$ allele (2) have been treated in culture with Ad-Cre (or floxed), while the control primary MECs were mock treated with Adenovirus-GFP. A transduction rate of 75-80% was achieved by counting ß-galactosidase positive cells prepared from ROSA26 reporter mice in the same experiment (1, 3).

The transduced primary MECs were harvested and injected into cleared fat pads of 21day-old nude mice. 7/7 of control primary MECs (not floxed) were able to reconstitute mammary glands, while 0/7 of floxed primary MECs grew out.

Figure 1.1.B



PCR on the reconstituted mammary glands show that in the AdCre treated floxß-catenin transplants, recominbination in exon 3 has occurred. (DNA recominbation PCR primers are obtained from Harada et al., CANCER RESEARCH 64, 48–54, January 1, 2004).

To summarize, stabilizing ß-catenin using the AdCre seems to inhibit mammary gland outgrowths. This data suggests that over-expressing ß-catenin in primary MECs inhibits the stem cells in the primary MECs from giving rise to differentiated cells necessary for reconstituting a mammary gland. It is possible that ß-catenin is important in stem cell survival, however, may also have an inhibitory effect when over-expressed. The lack of outgrowths from the floxed cells is not due to the Ad-Cre. The toxicity from the Adenovirus is minimal. Primary Wild Type (WT) MECs treated with Ad-Cre grew out as efficiently as WT MECs without Ad-Cre.

2. An alternative has been opted to analyze the effect of stem cell renewal – in vitro mammosphere assays.

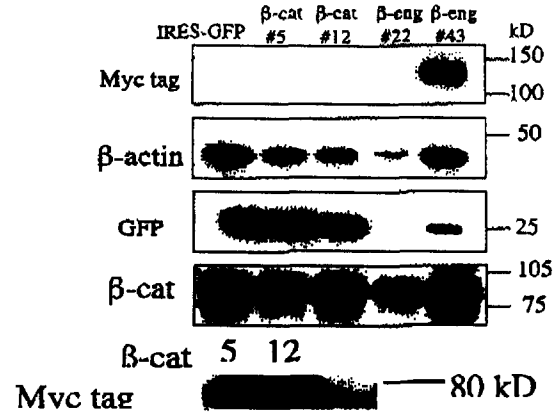
We initially proposed to analyze the effects of stem cell renewal by in vivo serial tissue transplants derived from the primary mammary gland reconstitution experiment. However, since the floxed primary MECs failed to reconstitute a cleared fat pad, the original proposal of serial tissue transplants in vivo would not work. Instead, we have opted to use an in vitro mammosphere assay to study the effect of stem cell renewal (4).

Analogous to neurospheres, mammary epithelial cells in suspension form clusters of cells that have been demonstrated to display an increased capacity for self-renewal. Using retroviral marking, It has been well characterized that mammospheres result from clonal expansion rather than cellular aggregation. They express stem cell markers such as CD49f, cytokeratin 5 and CD10; they contribute 100% to the SP fraction; and are able to differentiate into all cell types that make up the mammary gland. In addition, the secondary and tertiary mammospheres have been shown to be more homogeneous and are further enriched in self-renewal capacity.

To carry out the in vitro mammosphere experiment, we have done the following: First, a stabilized ß-catenin mutant and a ß-catenin dominant negative mutant (ß-eng) originally obtained from Dr. Pierre McCrea at U.T. M.D. Anderson Cancer Center in Houston, TX were subcloned into a retroviral backbone, mouse stem cell virus-IRES-GFP (MSCV-IRES-GFP) (5). The constructs of interest were packaged into retroviral particles in the presence of retroviral packaging proteins. The virus containing media were collected and used to transduce MECs.

Figure 1.2.A

Western Blot Analysis of Comma-D Transduced with β-cat, β-eng or IRES-GFP Only



To determine whether the MSCV-ß-cat, ß-eng constructs are expressing the right products, we transduced Comma-D mammary derived cell line with the virus containing media. The cells were harvested 48 hr after transduction, and protein extracts were prepared and used to determine whether the constructs are expressed on a Western Blot.

Figure 1.2.B

GFP Mammospheres are Cd49f+

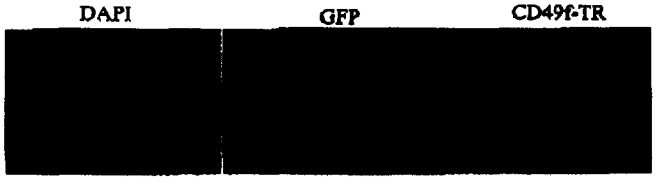
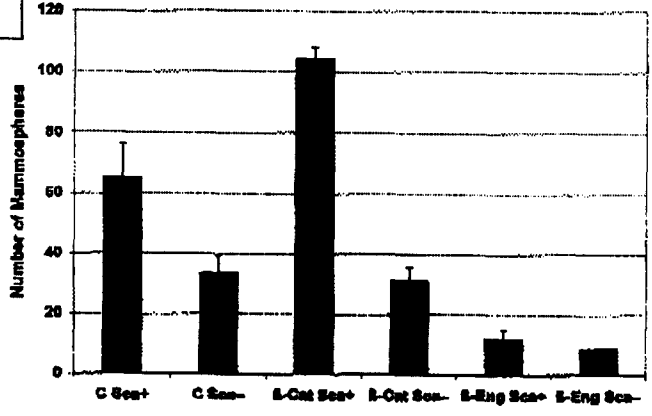


Figure 1.2.C **GFP Mammospheres are TIE-2+**

Following retroviral transduction, the MECs were plated onto a non-adherent surface, and grown in suspension for 7-10 days. The mammospheres were stained positive for potential surrogate stem cell markers CD49f and TIE-2, suggesting that they are enriched in stem/progenitor cells.

To look at the impact of the Wnt/ß-catenin pathway, we utilized COMMA-D (CD) cells. The CD cell line originally isolated from the midpregnant mammary gland of Balb/c mice have been shown to retain stem cell characteristics. These cells maintain both Side Population (SP) (0.1-1%) and Stem cell antigen 1 (Sca-1) (5-9%) populations in culture; and are able to differentiate and produce normal and preneoplastic mammary outgrowths when transplanted into mammary fat pads of syngeneic mice. In addition, Sca-1 is employed as a surrogate stem cell marker to separate the Sca-1+ from the Sca-1– cells as described by Welm et al.

Figure 1.2.D



CD cells were first transduced with a stabilizing mutated ß-catenin construct cloned into murine stem cell virus (MSCV) vectors with IRES-GFP tags (ß-cat), a dominant-negative ß-engrailed construct cloned into the same vector (ß-eng), or the MSCV-IRES-GFP vector alone as a control (GFP); and then sorted into Sca1+ and Sca1– populations. Since secondary generation mammospheres have been shown to be enriched in stem cell potential compared to first generation mammospheres, we have analyzed Sca+ and Sca– secondary mammospheres. The Sca1+ population demonstrated a significantly increased capacity for mammosphere formation compared to the Sca1– population (GFP control Sca1+ vs Sca1– 1.9 fold, p= 0.02; ß-

Cat Sca1+ vs Sca1– 3.3 fold, p= 6.88X10^8). In addition, CD cells transduced with stabilized ß-cat showed a significant increase in mammospheres compared to the GFP control (1.6 fold increase p= 0.008), while ß-eng, the dominant negative variant, showed decreased mammosphere formation compared to the control (5.4 fold decrease p= 0.27) . This data suggested that stabilizing ß-cat enriches for stem/progenitor cells while inhibiting ß-cat through the dominant negative ß-engrailed depletes stem/progenitor capacity for self-renewal.

3. The morphology by whole-mount, and histological analysis on the reconstituted mammary glands have been accomplished.

Figure 1.3.A

Floxß-cat Transplants H&E

Figure 1.3.B

Floxß-cat transplants do not stain for Ki67

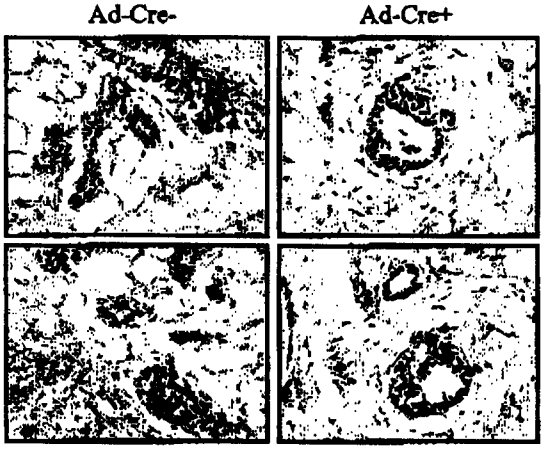
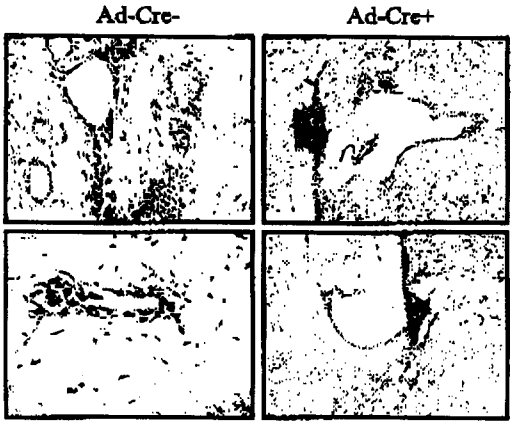


Figure 1.3.C

Floxß-cat transplants do not stain for cyclin D1



Mammary gland morphology was analyzed in detail in the control and the floxed transplants 8 weeks after transplantation. The floxed transplants either did not grow out, or had very limited outgrowth lacking any obvious mammary gland structures. Histological analysis showed that the transplanted cells did organize into two layers containing luminal and myoepithelial cells. Immunohistochemical analysis using Ki67, a proliferation marker, indicated that the control cells are actively cycling, while the floxed cells are not proliferating at all. This was confirmed by using cyclin D1, where the Ki67 positive control cells were also positive for cyclin D1, whereas the floxed cells that are negative for Ki67 are also negative for cyclin D1. The H&E of the floxß-cat transplants suggest that primary MECs expressing stabilized ß-catenin are able to form two distinct cell types: luminal and myoepithelial cells. Using the proliferation markers Ki67 and cyclin D1, we observed that the cells from the floxß-cat transplants are not proliferative when compared to the control transplants. This suggests that the floxß-cat transplants are somehow arrested in their development, and further experiments need to be done to delineate at what stage of the transplant the cells stop proliferating. However, if ß-catenin is inhibiting the differentiation of the stem cells, it is expected that the cells are unable to form a entirely reconstituted mammary gland upon reconstitution.

❑ Task#2

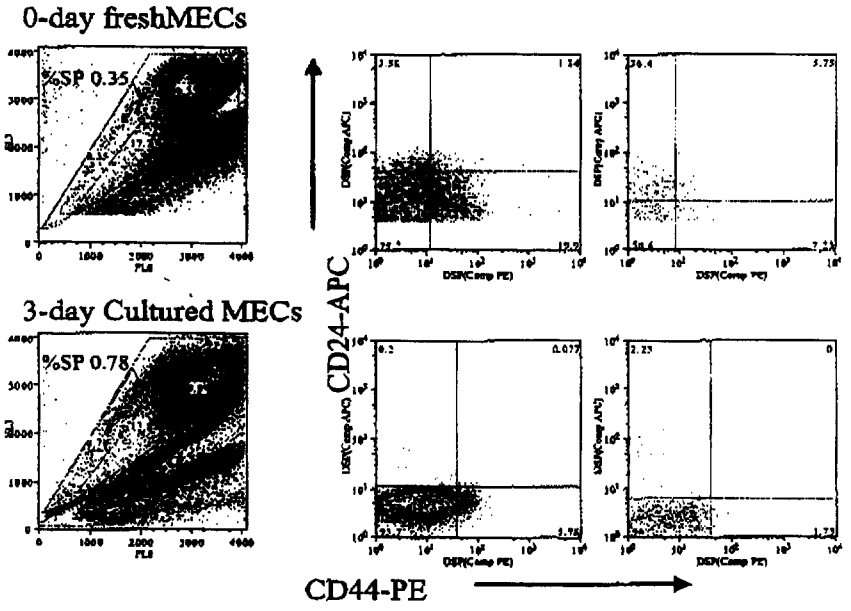
1. In depth FAC analysis has been carried out to profile changes in the stem/progenitor cell populations in MECs that express stabilized ß-catenin.

We chose to use the side population (SP) cells, which efflux Hoechst dye, as a surrogate marker of stem cells (6). Consistently, cells expressing the stabilized mutant ß-catenin either by using the loxP/Adeno-Cre, or by overexpressing the stabilized ß-catenin using MSCV- contained more SP cells than controls up to two fold.

2. Surrogate stem cell markers has been analyzed using flow cytometry.

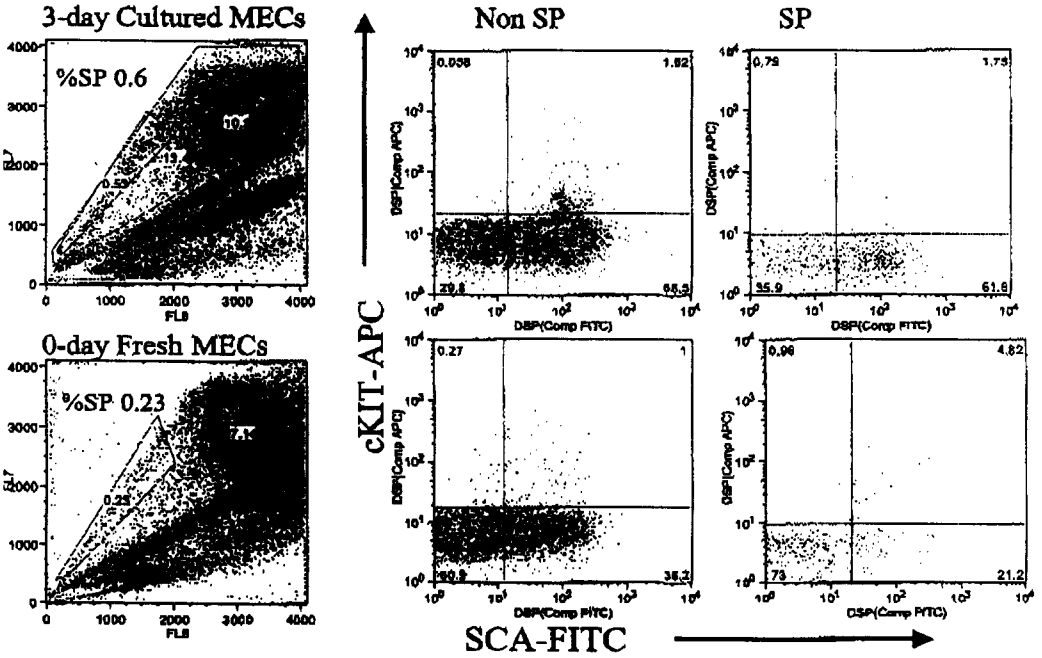
CD44+/CD24- population expands after 3-day culture

Figure 2.2.A



SCA1 expands in primary MECs culture, cKIT is low or negative

Figure 2.2.B



CD44+/CD24- has been used as markers for human breast tumor stem cells (7). In primary mouse MECs cultured at 0-day, MECs were $CD44^{+}/CD24^{high}$. After culturing for 48hr, the MECs are $CD44^{+}/CD24^{-/low}$. SCA1, another surrogate marker (6, 8), was high in primary mammary epithelial cells, and %SCA1+ cells increased in both SP and non-SP population after 48hrs of culture. CKIT, a surrogate stem cell marker used in hematopoetic studies, was very low or negative in primary MECs.

We have observed an expansion of SP when primary MECs are grown in culture. This may be due to the activation of the multi-drug pumps such as ABCG2 that are upregulated in the presence of growth factors in the culture media. However, the stem/progenitor potential within the cultured primary MECs do not appear to be compromised by culturing, since we are able to use the cultured primary MECs for all of our transplant experiments. CD44+/CD24– has been used by Wicha et. al. as cancer stem cell markers (ref. Al-Hajj et. al., Proc Natl Acad Sci U S A. 2003 Apr 1;100(7):3983-8). We have observed that both CD44 and CD24 are present in the WT primary MECs, although data in our lab indicates that CD24 appear to be present at higher levels in tumor cell lines (not shown). How CD44+/CD24– in the WT MECs behave compared to the cancer stem cell markers is not known. Experiments are on-going to determine the clonogenic and perhaps tumorigenic potential of these CD44+/CD24– cells. The other two potential stem cell markers that we have examined are Sca-1 and cKIT. Both have been used extensively in the hematopoietics field to identify bone marrow stem cells. We have observed that primary MECs express Sca-1, but not cKIT. The reason we are not able to find cKIT positive cells may be due to the inherent cell prepration procedure which chews up cKIT's labile surface marker. The advantage of employing Sca-1 as an alternative stem cell marker is that we can use it to isolate stem/progenitor cells without the toxicity of the Hoechst dye.

Task#3 To determine if ß-catenin overexpression decreases the sensitivity to apoptosis by treating primary MECs expressing stabilized ß-catenin with radiation. Please see attached figures following page 14.

To test the hypothesis that SP/stem-like progenitor cells in the mammary gland are resistant to radiation compared to the non-SP populoation, we irradiated cultured primary MECs from Balb/c mice using 137Cs γ-irradiation and analyzed the percentage of SP cells, in the total population after treatment. Irradiation of Babl/c MECs in culture lead to a 4-fold increase in the %SP at 2 Gy. The increase in %SP decreased at 6 Gy, but was still over 3-fold higher than baseline.

To examine the role of the putative mammary stem cell survival factor Wnt/ ß – catenin in mediating radioresistance of SP cells, MECs from C57BL/6 transgenic mice containing a floxed allele of exon III of ß-catenin (22) were isolated and transduced with an adenovirus encoding Cre recombinase (AdCre) or a comparable titer of a control adenovirus encoding ß-galactosidase (AdLacZ) on day 3 of culture. Cells were irradiated on day 4 and stained with Hoechst 33342 for SP analysis 24 hours later. PCR was used to demonstrate efficient recombination (>75%) in the primary MEC cultures transduced with AdCre (data not shown). Primary MECs containing stabilized ß-catenin exhibited a greater radiation-induced increase in %SP (0 vs. 2 Gy $P = 0.02$, 0 vs. 4 Gy $P = 0.04$) than AdLacZ treated controls (Figure 1b). Interestingly, the radiation-induced increase in the %SP in the wildtype C57BL/6 mice was less remarkable than that observed in MECs

from Balb/c mice, consistent with previous studies demonstrating increased radiosensitivity of the mammary epithelial cells in Balb/c mice compared with various other strains of inbred mice.

The impact of increased Wnt signaling on radioresistance of SP cells was also examined using MECs isolated from mouse mammary tumor virus (MMTV)-driven Wnt-1 transgenic mice at 10-12 weeks of age treated as described above. MECs isolated from Wnt-1-induced hyperplasias exhibited a greater radiation-induced increase in %SP than MECs isolated from mice of a matched background ($P = 0.08$, Figure 1c). Consistent with similar data reported by Lui et. al., we observed the %SP in MECs from Wnt-induced hyperplasias to be significantly higher (> 2 fold) than in background matched controls (%SP 0.75 vs. 0.32, $P = 0.03$).

To examine the effect of Wnt/ß-catenin in an immortalized cell line that retains a heterogenous cell population and can give rise to hyperplastic mammary outgrowths upon transplantation, we employed the COMMA-D (CD) cell line. The CD cell line originally isolated from the midpregnant mammary gland of Balb/c mice have been shown to retain stem cell characteristics. These cells maintain both SP (0.1-1%) and Sca1 (5-9%) populations in culture; and are able to differentiate and produce normal and preneoplastic mammary outgrowths when transplanted into mammary fat pads of syngeneic mice. To demonstrate that the CD stem-like/progenitor cells are able to self-renew and differentiate we have employed a number of approaches to delineate its stem cell potential.

To examine the stem cell potential of CD stem-like/progenitor cells, we have compared the self-renewing potential of progenitor CD and non-progenitor cells using Sca1 as a surrogate stem/progenitor marker. The CD cells generally expressed 7.2 ± 2.5% Sca1 in culture (Figure 2a). We sorted the cells into Sca1+ and Sca1– populations, and analyzed the Sca1 distribution after culturing for 72 hrs. The Sca1+ cells were able to give rise both Sca1+ as well as Sca1– cells after culturing. The Sca1– cells, however, remained mostly Sca1– after culturing (Figure 2b). The original 7.2% Sca1+ cells were bi-potent, giving rise to both 75% ± 4.2 Sca1+ and 24% ± 4.1 Sca1– cells while the Sca1– cells gave rise to 94% ± 1.5 Sca1– cells and very few, 6% ± 1.45 Sca1+ cells (Figure 2c).

Next, we looked at the clonogenicity of CD Sca1+ cells. Clonogenic cells are defined as those neoplastic cells within a tumor that have the capacity to produce an expanding colony of descendants, and therefore, the capacity to regrow the tumor if left intact at the end of treatment. Since the CD cells are known to carry a mutant p53 allele, and are able to give rise to hyperplastic outgrowths containing both ductal and alveolar structures in mammary gland reconstitution experiments, we considered its capacity to form clonogens. We sorted the cells into Sca1high (7.8%), Sca1medium (12.3%) and Sca1low (76.4%) populations based on the amount of Sca1 fluorescence (Figure 2a). The three populations were directly sorted into 96 well plates containing Matrigel in equal numbers, and their clonogenicity were assessed after 10 days. The Sca1high cells were the most clonogenic, giving rise to 47± 14 colonies in Matrigel, followed by the Sca1medium population, which gave rise to 15± 5.6 colonies, while the Sca1low population failed to produce any colonies (2d).

To further establish that the Sca1+ CD cells have the potential to self-renew and to examine the impact of the Wnt/ß-catenin on stem/progenitor self-renewal, we adapted the

mammosphere culture to assay for self-renewal in the Sca1+ and Sca1– CD cells. Analogous to neurospheres, mammary epithelial cells in suspension form clusters of cells that have been demonstrated to display an increased capacity for self-renewal. Using retroviral marking, It has been well characterized that mammospheres result from clonal expansion rather than cellular aggregation. They express stem cell markers such as CD49f, cytokeratin 5 and CD10; they contribute 100% to the SP fraction; and are able to differentiate into all cell types that make up the mammary gland. In addition, the secondary and tertiary mammospheres have been shown to be more homogeneous and are further enriched in self-renewal capacity.

CD cells cultured in suspension were able to form mammospheres, and expressed stem cell markers such as CD49f and TIE-2 (data not shown). To look at the impact of the Wnt/ß-catenin pathway, CD cells were first transduced with a stabilizing mutated ß-catenin construct cloned into murine stem cell virus (MSCV) vectors with IRES-GFP tags (ß-cat), a dominant-negative ß-engrailed construct cloned into the same vector (ß-eng), or the MSCV-IRES-GFP vector alone as a control (GFP); and then sorted into Sca1+ and Sca1– populations. Since secondary generation mammospheres have been shown to be enriched in stem cell potential compared to first generation mammospheres, we have analyzed Sca+ and Sca– secondary mammospheres. The Sca1+ population demonstrated a significantly increased capacity for mammosphere formation compared to the Sca1– population (GFP control Sca1+ vs Sca1– 1.9 fold, p= 0.02; ß-Cat Sca1+ vs Sca1– 3.3 fold, p= 6.88X10^8) (Figure 3a). In addition, CD cells transduced with stabilized ß-cat showed a significant increase in mammospheres compared to the GFP control (1.6 fold increase p= 0.008), while ß-eng, the dominant negative variant, showed decreased mammosphere formation compared to the control (5.4 fold decrease p= 0.27) . This data suggested that stabilizing ß-cat enriches for stem/progenitor cells while inhibiting ß-cat through the dominant negative ß-engrailed depletes stem/progenitor capacity for self-renewal.

We hypothesized that if Wnt/ß-catenin signaling can function as a stem cell survival factor, stabilized ß-catenin might enhance mammary outgrowths from transplanted cells, while inhibition of ß-catenin signaling through expression of the dominant-negative construct ß-engrailed might inhibit or delay outgrowth. To examine the effect of the ß-catenin signaling on the ability to form outgrowths we transduced CD cells with the ß-galactosidase (control), stabilized ß-catenin, and ß-engrailed constructs cloned into pS2 vectors previously described (28) and transplanted these cells into the cleared fat pads of 3 week-old Balb/c mice. Eight fat pads (4 mice) were transplanted per construct. An initial biopsy (n = 2 per construct) was taken for whole mount staining at 8 weeks. All mice were sacrificed by 28 weeks and all outgrowths were imbedded for pathology. All slides were reviewed with a breast pathologist with expertise in mouse mammary development.

Outgrowths from mice transplanted with ß-catenin-transduced cells were consistently more prominent and filled a larger percentage of the fat pad (Figure 3b); (eight-week outgrowths). Although a small number of tumors developed from cells transduced with each of the three constructs by twenty weeks, ß-catenin-derived outgrowths which produced tumors developed consistently invasive, high-grade tumors, and all ß-catenin derived outgrowths after 8 weeks demonstrated evidence of mammary intraepithelial neoplasia (MIN, Figure 4 - ß-catenin H&E inset). In preliminary experiments, an

increased survival fraction was observed in irradiated cells derived from ß-catenin-induced tumors as compared to those from the control ß-galactoside transduced CD cells (data not shown).

Clonogenic assays are classically used to demonstrate radioresistance, since cells that can form colonies after radiation are clearly still competent to reproduce and, therefore, represent a fraction of cells that can recur and lead to tumor recurrence. These assays are usually not feasible in primary cultures, since it is difficult to maintain these cells in culture long enough to form colonies. These assays are also of limited utility after SP analysis, because of the toxicity of Hoechst dye in combination with the trauma of cell sorting. In this setting the plating efficiency of these cells can be as low as 1: 1000. To overcome this limitation, we compared clonogenicity of progenitor CD cells after radiation to non-progenitor CD cells using Sca-1 to identify progenitor cells as described by Welm et al (7). Immortalized CD cells were irradiated and labeled with a FITC conjugated Sca-1 antibody. Sca-1 positive (Sca1+) and negative (Sca1-) cells were sorted into 96 well plates containing Matrigel in the center of the well (Figure 4a). 7 +/- 2% of untreated CD cells were Sca1+. Plating efficiency (number of colonies/number of cells plated) for colony formation from Sca1- cells was 0% while plating efficiency for colony formation from Sca1+ cells was 2-4%, therefore Sca1- cells were unable to self-renew and form colonies in Matrigel. The Sca1+ surviving fraction after 2 Gy was 100% (Figure 4b).

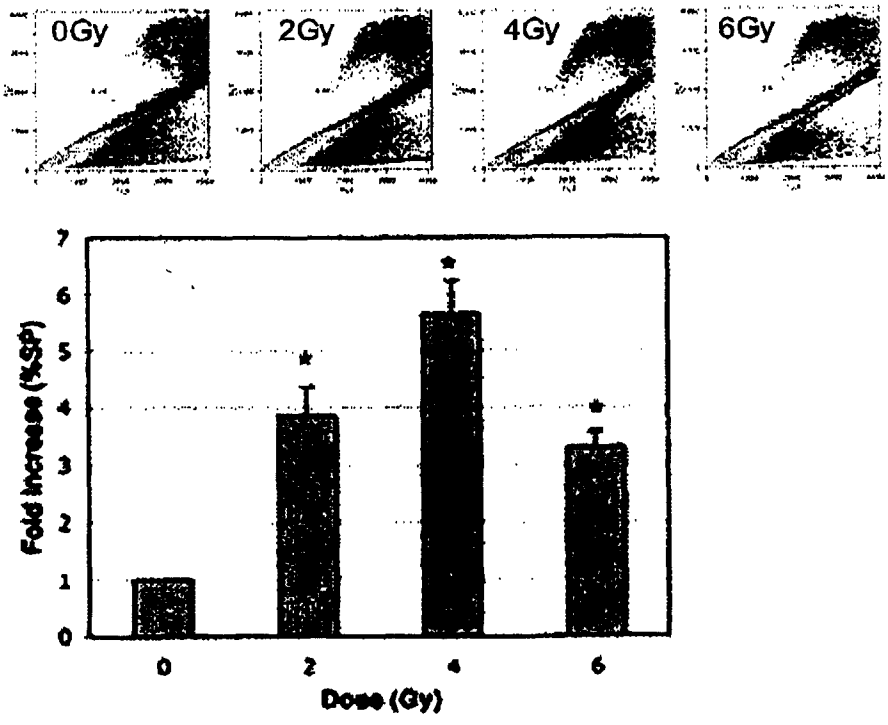
To test the hypothesis that the progenitor cells are more resistant to clinically relevant doses of ionizing radiation, we analyzed the long-term effects of radiation on the survival of Sca1+ and Sca1– cells using an established cell proliferation assay. CD cells were sorted into Sca1+ and Sca1– populations using a FITC-conjugated Sca1 antibody. After sorting the cells were irradiated at 0, 2, 4 and 6Gy, and then plated. MTT assay was used to compare cell proliferation between the Sca1+ and Sca1– cells at 24hr, 48hr, and 144hr. 144hr following irradiation, we observed a significant difference in proliferation between the Sca1+ and Sca1– cells with equivalent doses (0Gy, 4 fold; 2Gy, 7 fold; 4Gy, 8 fold; 6Gy, 5 fold; $p<0.05$, two-tailed T test). No significant difference was observed at 24hr or 48hr time points. There were no significant difference in cell death between the Sca1+ and Sca1– cells at 24hr, 48hr, and 144hr in response to radiation (data not shown).

Recent studies have shown that activation of the pathway enriches for stem/progenitor cells, which are thought to correlate with increased cancer risk (ref. Polakis). To examine the effect of stabilized ß-Catenin in stem-like/progenitor expansion following radiation, we utilized CD cells that were transduced with GFP control, ß-cat or ß-eng. Cells transduced with stabilized ß-catenin exhibited a radiation-induced expansion in %SP (2 Gy vs 0 Gy,1.7fold), while cells transduced with dominant-negative ß-engrailed did not (Figure 4c.). The expansion of %SP in CD cells expressing stabilized ß-catenin is consistent with the observations we made in the primary MECs, suggesting that Wnt/ß-catenin is responsible for the expansion of stem/progenitor cells following radiation. Previous data from our laboratory have shown that expression of the dominant-negative construct ß-engrailed leads to marked apoptosis in MECs. This increased propensity for apoptosis in ß-engrailed expressing cells, and the presence of endogenous ß-catenin in cells expressing low levels of ß-engrailed prevented us from directly demonstrating their increased sensitization to radiation in the MSCV-ß-engrailed transduced cells.

This study is the first to demonstrate that stem-like progenitor cells in the mammary gland can be more resistant to clinically relevant doses of radiation than non-stem-like progenitors, which constitute the bulk of the mammary gland. We also demonstrated that stem-like progenitors in the immortalized CD cell line are also resistant to radiation, and radiation resistance in both MECs and CD cells can be altered through manipulation of the Wnt/ß-catenin stem cell survival pathway. These data are of clinical importance because radiation therapy is a valuable component of breast conserving cancer therapy, and improves overall survival in selected patients treated with mastectomy. Despite radiation, surgery, and chemotherapy, a number of patients still have disease recurrence and there remains considerable need for improvement in treatments. Our data suggest that targeting cancer stem cells may offer a new strategy for sensitizing breast cancers to radiation. It may be possible to improve locoregional control and ultimately survival in breast cancer through direct targeting of stem cell survival pathways such as Wnt/ß-catenin during radiation.

Figure 1

a.



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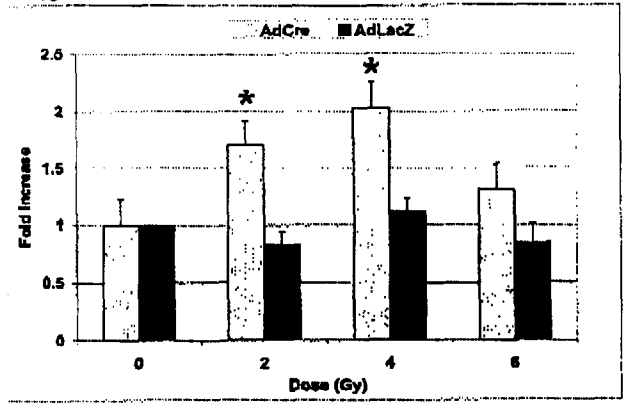


c.

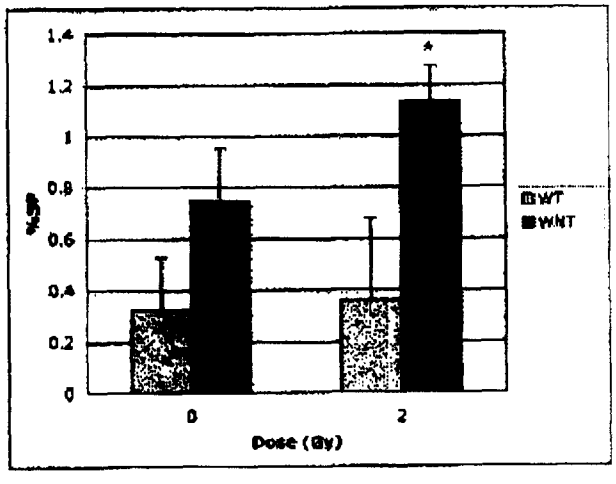


Figure 2

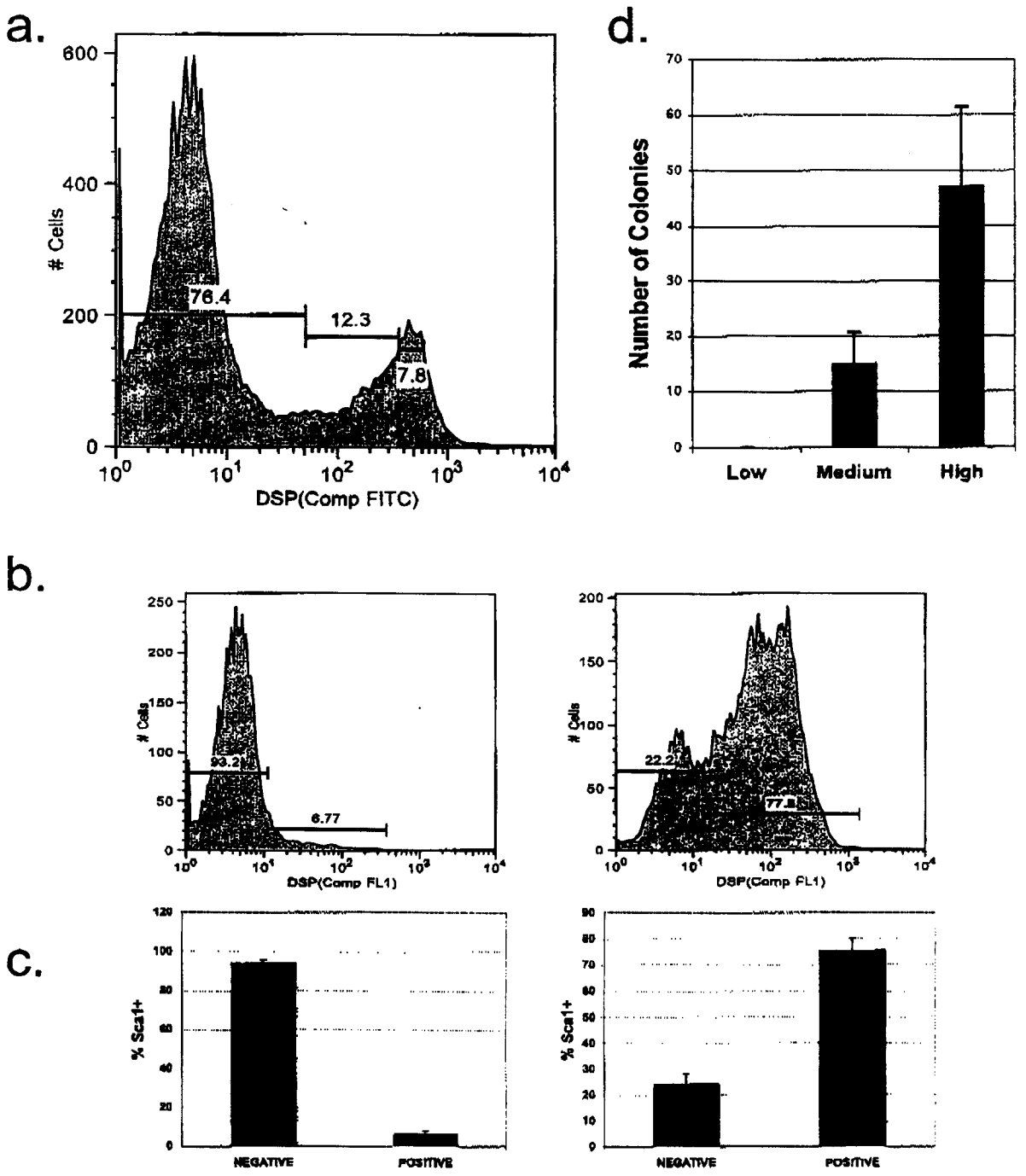
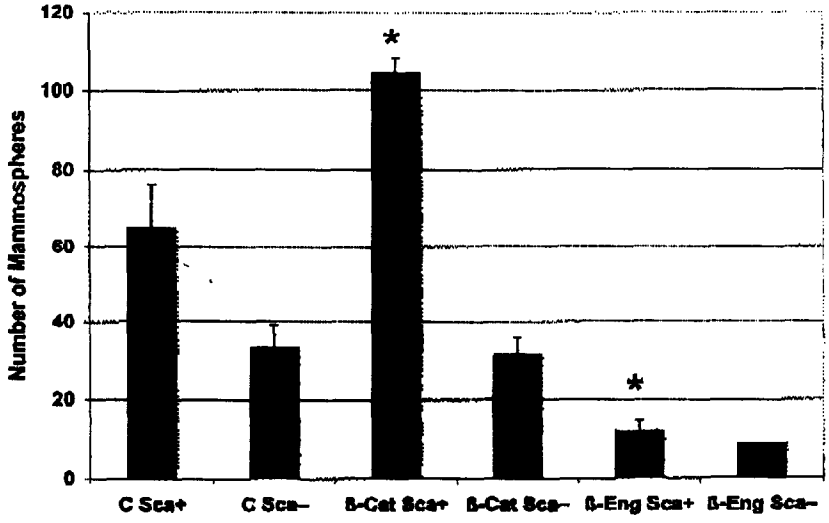


Figure 3

a.



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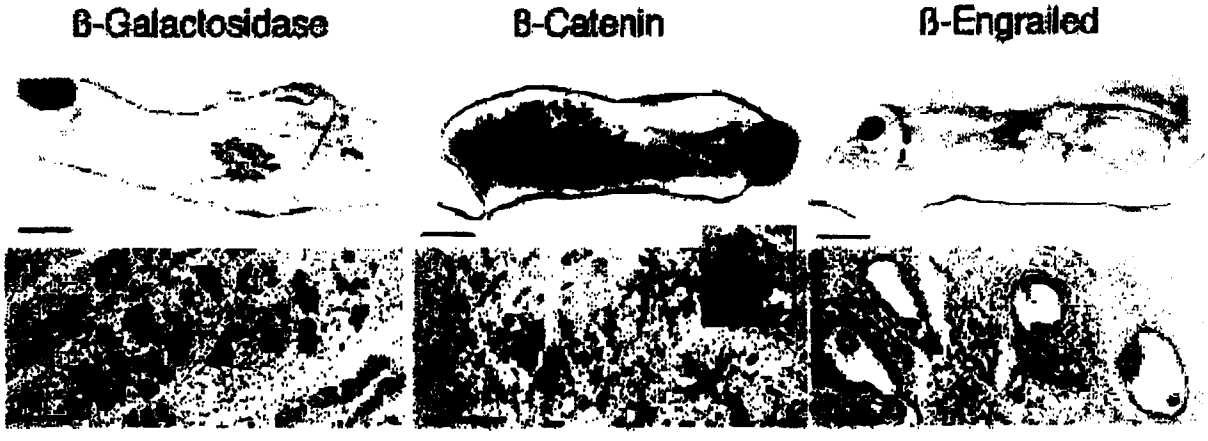
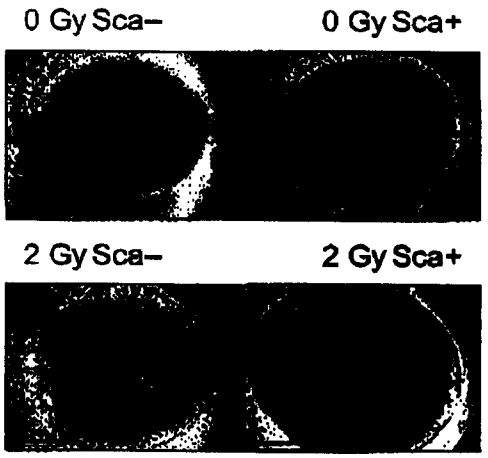


Figure 4

a.



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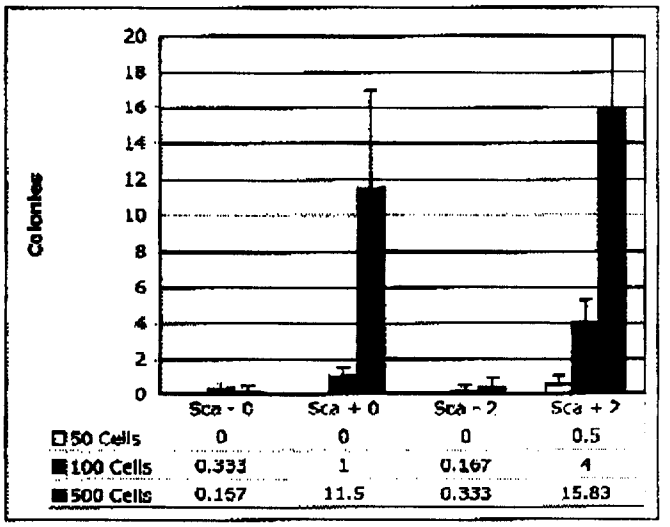

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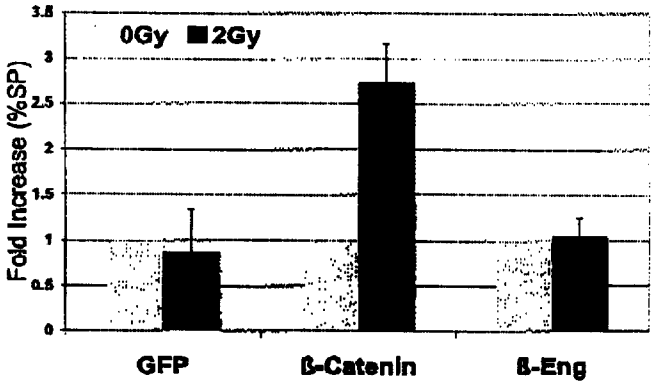

Key Research Accomplishments:

Poster Presentation:
Mercy S. Chen, Wendy A. Woodward, Jeffrey M. Rosen. ß-catenin: A pivotal Role in Mammary Gland Stem Cell Survival and Differentiation. Gordon Research Conference in Mammary Gland Biology, May 2004

Manuscript:
Wendy A. Woodward, Mercy S. Chen, Fariba Behbod, Maria P. Alfaro, Thomas Buchholz, and Jeffrey M. Rosen. Wnt/ß-catenin-Mediated Radiation Resistance of Mouse Mammary Stem-like/Progenitor Cells. (Manuscript in preparation.)

Reportable Outcomes:

Constructs:
The ß-catenin and ß-eng cloned into MSCV-IRES-GFP backbone will be valuable to our lab and others for analysis of ß-catenin function.

Methods developed:
Using the IRES-GFP tag to facilitate flow cytometry sorting and analysis of cells by enriching for cells that express the IRES-GFP and the protein of interest, either ß-catenin or ß-eng.
Transducing mammospheres with retroviral constructs and adapting the in vitro mammosphere assay to COMMA-D cells as an assay for stem cell self-renewal and proliferation.

Conclusions:

We have modified techniques to allow us to answer the question of stemness and the role of ß-catenin in stem cell renewal. We are on the right track with respect to the assigned tasks, we have reported a number of fascinating observations here, and we are also preparing a manuscript that pertains to Task #3. Overall, we are pleased with the progress we have made in the past year, and we fell confident that we will complete the remaining tasks on schedule.

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